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# GREEN MEAT OF SWORDFISH

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Frozen swordfish is one of the most important marine products of Japan. Its exports, which have been on the increase since 1947, rose to 5223 short tons in 1951. The largest market is the U. S. A.

The so-called green meat of swordfish has been noticed with alarm for some years in the swordfish industry; the normal white meat turns greenish giving off an offensive odour. Commercial grading procedures disqualify the meat so affected. Such unfavorable cases steadily increased as the amounts of the catch and export of swordfish increased, thereby incurring considerable losses to the dealers of the fish. Naturally they are anxious to prevent the occurrence of such a change in the fish meat. However, the literature on this subject is found very meagre. Y. Amano (1949) suggested that the green meat may occur as the result of combination of hydrogen sulfide and an unknown substance in the muscle and that one of the odour principles is isovaleric acid.<sup>1)</sup> Y. Arai and his collaborators (1949) isolated 12 cultures of *Pseudomonas* from the green meat of swordfish and holds of fishing boats but they could not get any artificial green meat by means of the inoculation of those bacteria in normal meat.<sup>2)</sup> Y. Tsuchiya and his collaborators (1953) found that the green meat frequently occurs more on the down side of the fish facing the floor after it is caught.<sup>3)</sup>

This paper deals with the results of experiments conducted by the present authors on the chemical natures of the green meat of swordfish.

## Experiment

### *Materials*

Materials used in this experiment were normal and green meat of swordfish (*Xiphias gladius* Linné) landed at Kesennuma, Miyagi Prefecture, in summer of 1952.

### *Hydrogen sulfide and volatile basic nitrogen*

As the green meat gives off a stale odour, the determination of hydrogen sulfide and volatile basic nitrogen of meat was made. Hydrogen sulfide was estimated

according to the method of L. H. Almy.<sup>4)</sup> Volatile basic nitrogen was determined by the method of Conway.<sup>5)</sup> The results are given in Table 1.

**Table 1.** Hydrogen sulfide and volatile basic nitrogen in swordfish meat.

	Hydrogen sulfide (mg %)		Volatile basic nitrogen (mg %)		
	I	II	I	II	III
Green meat	1.23	1.34	29.96	23.04	—
Normal meat	0.37	0.83	16.26	14.03	13.55

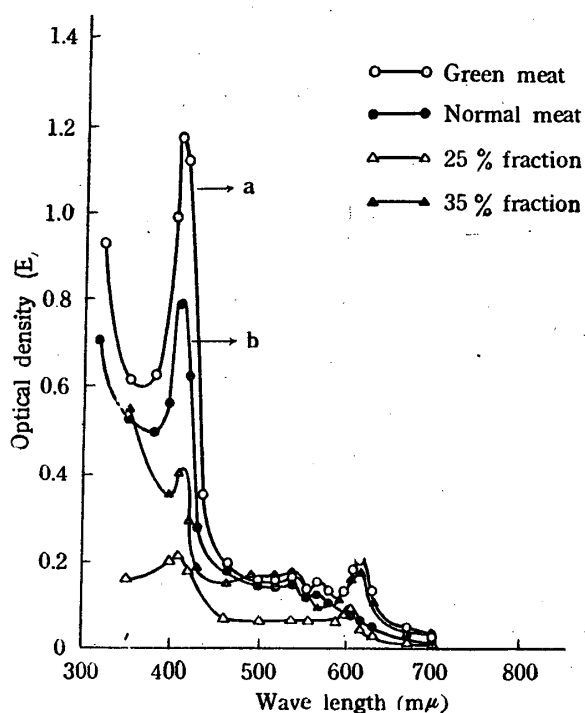
Above table shows that the green meat contains a larger amounts of hydrogen sulfide and volatile basic nitrogen than the normal meat, and suggests that the discoloration may have some relations to a spoilage of the meat.

#### *Chemical nature of green pigment*

To examine the green pigment, the following procedure was adopted. Place one hundred grams of green meat into a mortar, thoroughly grind it and then mix with 100 ml. of water. After allowing to stand for about one hour at room temperature, clear filter off the solution. The filtrates were fractionally precipitated with the addition of ammonium sulfate. The green pigment

precipitates at the higher concentration than 25 per cent ammonium sulfate. Each fraction was dissolved in water and spectrophotographed by Beckman spectrophotometer. The water extracts were also examined directly without above procedures. The results obtained are shown in Figure 1.

**Fig. 1** The absorption curves of water extract of swordfish meats.



It is found that the absorption curve of green pigment has a distinct maximum absorption band at 620  $m\mu$  which is not recognized on that of normal meat. This absorption maximum indicates the characteristic spectrum of sulfhemoglobin which is a green compound produced by the combination of hydrogen sulfide and hemoglobin under the existence of oxygen. Thus, the

green meat of swordfish was found as attributable to the formation of sulfhemoglobin in the muscle.

Next, the green pigments were subjected to the current of carbon monoxide and then the spectra of the solution were observed as shown in Figure 2.

It is found by H.

O. Michel already that when sulfhemoglobin combines with one mole of carbon monoxide, the absorption band of carboxy-sulfhemoglobin formed shifts to shorter wave length (612.... 618  $m\mu$ ).<sup>6)</sup> It shows that this figure does exactly fit with the spectrum of carboxy-sulfhemoglobin.

#### *Isolation of hydrogen sulfide-producing bacteria*

As the discoloration of swordfish meat is mainly due to the production of sulfhemoglobin, it is assumed that hydrogen sulfide-producing bacteria will play an important role in the formation of green pigment in the muscle.

A total of 23 microorganisms were isolated from frozen and fresh green meat by their figure and colour of colony. Then they were inoculated to bouillon containing 0.1 per cent cystine and incubated at 36°C and the production of hydrogen sulfide was tested by lead acetate paper. The results are given in Table 2.

Table 2 shows that 4 of 23 microorganisms isolated from the green meat of swordfish have a power to produce hydrogen sulfide. Although the inoculation tests for hydrogen sulfide-producing bacteria to the normal meat were tried under many conditions with special considerations on varied temperatures and times for the growth of the bacteria, we have failed to get any artificial green meat.

Fig 2. Effect of carbon monoxide on the absorption curve of green pigment

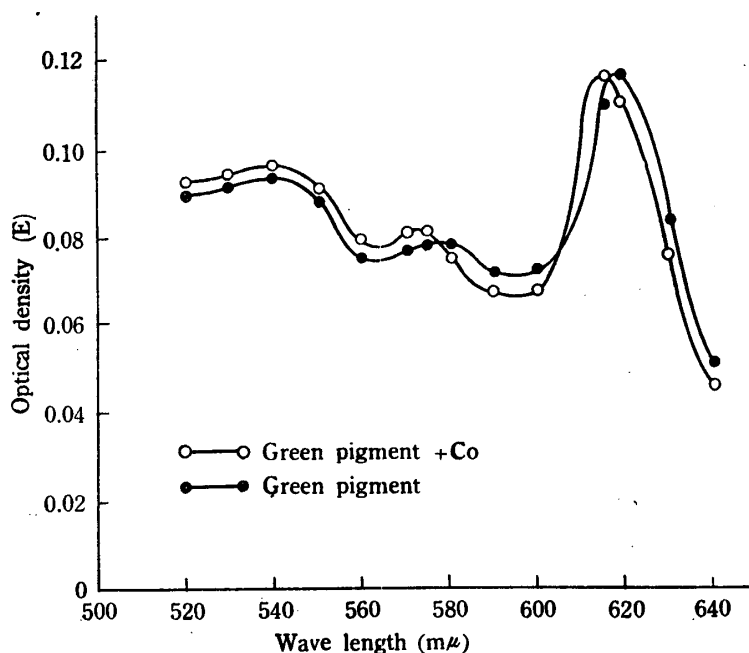


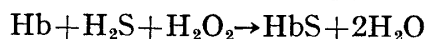
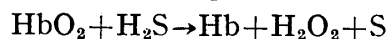
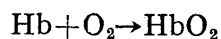
Table 2. H<sub>2</sub>S productivity of microorganisms.

Culture number	Incubation time in days				
	1	2	3	5	30
A-1	-	-	-	-	-
2	-	-	±	++	+
3	-	-	-	-	±
B-1	-	-	-	-	±
2	-	-	-	-	±
3, 4	-	-	-	-	-
C-1, 2	-	-	-	-	-
D-1, 2, 3, 4	-	-	-	-	-
E-1	-	-	-	++	+++
2, 3, 4	-	-	-	-	-
5	-	-	-	++	+++
6	-	-	±	++	+++
7	-	-	-	-	±
8, 9, 10	-	-	-	-	-

Note :-        - indicates negative production  
                   + indicates positive production

### Discussion

As pointed out by H. O. Michel, a green compound sulfhemoglobin is formed by the combination of hemoglobin and hydrogen sulfide under the existence of oxygen as follows.<sup>7)</sup>



The hypothesis that this sulfhemoglobin is the main cause of green meat of swordfish seems to be proven by the following results of our studies.

1. It is found in our previous report that more green meat develops on the down side of a fish facing the floor than on the up side. This seems to suggest that putrid blood is more congested on down side than on the up side.

2. As is shown in curve a and b of Figure 1, the value of optical density for the extracts prepared by the same procedure is higher in green meat than in normal meat. We assume therefore that blood or hemoglobin is much more concentrated in green meat than in normal meat.

3. Green meat mostly develops in the outer part of the fish body instead of the inner part. This fact seems to indicate that the green meat requires a supply of air for its development.

4. Hydrogen sulfide-producing microorganisms were isolated from green meat, and in addition we found the fact that much more hydrogen sulfide was observable in green meat than in normal meat.

In consideration of these facts, it is concluded that the cause of green meat

of swordfish is mainly due to the sulphemoglobin produced by the putrefaction of blood or hemoglobin.

Furthermore, it is inferred that green meat may also be affected by sulfmyoglobin which occurs from red muscle pigment myoglobin under similar conditions. However, the maximum absorption of sulfmyoglobin is shorter (617 m $\mu$ ) compared with that of green meat. The swordfish is generally known as one of the white meat fish, and although it is not described here, it is significant to say that the isolation experiments of myoglobin from it have been unsuccessful. Thus, myoglobin in the meat seems to be very slight and consequently sulfmyoglobin have no effect on the development of green meat of swordfish.

For prevention of green meat of swordfish, the most effective method is suggested to be a complete bleeding and good preservation of the meat on aboard the fishing boat after catching. And at the same time, it is unquestionable that the fish must be handled quickly and sanitarily.

### Summary

1. Green meat of swordfish is attributable to a green pigment sulphemoglobin which is formed by the combination of hydrogen sulfide and hemoglobin under aerobic condition.
2. Four cultures of hydrogen sulfide-producing bacteria were isolated from green meat of swordfish.
3. General considerations on the possibility and mechanisms of sulphemoglobin formation and on the methods for prevention of green meat were made.

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